Development of Proteomics-Based Methods for Protein Quantification

Reference measurement procedures are an important part of clinical measurement standardization. They can be used to directly assess the accuracy of routine methods or can be used to assign or verify the concentrations of controls and calibrators used in routine methods. Reference measurement procedures also provide a means to demonstrate traceability of routine methods and materials to higher-order reference materials.

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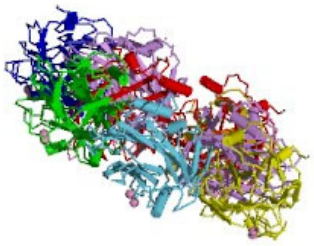
While there are many reference measurement procedures for clinical inorganic and small organic species, few exist for clinically relevant proteins. A recent survey of clinical reference methods by the Joint Committee for Traceability in Laboratory Medicine (JCTLM) indicated approximately 25 documented reference methods for serum proteins, most using immunological methodologies or an enzyme activity measurement. Only two reference methods, both for HbA1c, use modern analytical measurement procedures — such as capillary electrophoresis, liquid chromatography, and mass spectrometry — for the sensitive and direct measurement of the clinical analyte. In order to meet the standardization needs for clinically relevant proteins, new reference measurement procedures must be developed.

In this work, an approach typically used in "bottom-up" proteomics is used for the quantification of clinically relevant proteins in serum. Specifically, quantification is achieved through the measurement of peptides generated from the enzymatic digestion of the target protein in serum. Because the analyte (protein) and measurand (peptides) are different, care must be taken to identify potential sources of bias in the enzymatic digestion process that converts analyte to measurand. Therefore, much of the focus in the development of this approach has been in the exploration of the enzymatic digestion process.

Because proteolytic digests, particularly trypsin digests, are at the core of our proteomics-based approach, we have devoted a large effort to understanding the practical nature of trypsin through fundamental studies of how experimental factors effect trypsin digests of analyte proteins. In collaboration with scientists from the national metrology institutes of the United Kingdom (LGC) and Germany (PTB) we have explored the quantitative nature of tryptic digestion. Successful quantitative measurements have been made on serum C-reactive protein using this approach.



C-Reactive Protein
Source: *Protein Data Bank*

We have also investigated new data analysis algorithms, such as principle components analysis, to evaluate digestion reproducibility and the chemical and measurement factors that have the largest influence on reproducibility.

As the field of proteomics matures, it is very likely that more protein biomarkers will be discovered and used for clinical diagnoses. New immunoassays for protein biomarkers will require validation through more metrologically sound methods, such as the ones being developed using mass spectrometry.

Accurate and precise protein quantitation may help answer fundamental biological questions regarding protein expression and its relation to the genome and environment. Additionally, the techniques developed for protein quantitation can also be used in the areas of drug discovery and biotechnology.

Future Plans:
Because protein quantification is a critical measurement capability, NIST will continue research in the development of proteomic-based approaches to protein quantification. A new effort in the *in vitro* production of protein, specifically isotopically labeled proteins, will provide needed internal standards for mass spectrometric-based measurements.